HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

Vol. 11 | MAY, 1938 | No. 5

THE DEVELOPMENT OF RESISTANCE TO HYDROCYANIC ACID IN CERTAIN SCALE INSECTS[1, 2]

H. J. QUAYLE[3]

INTRODUCTION AND REVIEW OF LITERATURE

In 1914 Melander published an article under the title, "Can Insects Become Resistant to Sprays?" Two years later an article appeared under the title, "Are Scale Insects Becoming Resistant to Hydrocyanic Acid Fumigation?" (Quayle, 1916). Since the possibility that insects could develop a tolerance for sprays and fumigants was a new conception, the writers quoted put the titles to their first articles in the form of questions. With the accumulation of more evidence, the present writer became more confident, and six years later published an article entitled, "Resistance of Certain Scale Insects in Certain Localities to Hydrocyanic Fumigation" (Quayle, 1922); Melander, nine years after his first article appeared, published a second article entitled, "Tolerance of San Jose Scale to Sprays" (Melander, 1923).

Since that time other writers (Woglum, 1925; Gray and Kirkpatrick, 1929, *a*, *b*) have secured additional evidence on resistance in relation to fumigation; and members of the Citrus Experiment Station staff and other workers whose investigations are related to the question have secured ample evidence to support what the writer now considers a well-established fact.

There are still, however, dissenting opinions. Moore (1933, p. 1161) states, "Under favorable conditions, there is no significant difference between the kills of 'resistant' and 'nonresistant' red scale. The main difference between resistant and nonresistant red scale is the influence, upon

[1] Received for publication October 13, 1937.

[2] Paper No. 346, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Professor of Entomology in the Citrus Experiment Station and Graduate School of Tropical Agriculture and Entomologist in the Experiment Station.

the results of fumigation, of certain environmental conditions." Cunningham (1935, p. 105) states, "When one considers the complexity of the problems involved in tent fumigation, it becomes obvious that many points must be investigated before the theory of 'resistant' scales can be accepted." Many others who have not worked on the problem and consequently have not published their opinions or conclusions, nevertheless doubt that such a phenomenon as insect resistance to insecticides has developed. This attitude may be understood, for the published data supporting the fact have been very limited. The object of the present article is to make known the data secured by other investigations as well as the writer's. The question has a very important bearing on the broad subject of the use of insecticides. Specifically, it has been the direct cause of the most serious insect-control problem that has confronted the California citrus industry in the past fifty years, or since the discovery of hydrocyanic acid fumigation.

Meaning of Resistance.—The term "resistant scale" as here used means scale that, while not immune to the fumigant, requires such high dosage for satisfactory results in practical fumigation that the tree is likely to be injured. "Nonresistant" scales succumb to fumigation dosages that are usually safe for the tree. In practice, fumigation is expected to effect such a high mortality on scale insects that treatment ordinarily is not again necessary for two or more years. Such results are still being secured in the greater portion of the citrus areas of California, where it is recognized that the insects are not difficult to kill. On the other hand, in those areas where it is recognized that the insects are difficult to kill, or are resistant, the results of fumigation are such that the trees may not remain clean for a single year. In such cases two annual fumigations are necessary, or the application of an oil spray followed by fumigation.

The term "resistant scale" as used in this discussion may not be the best designation, but the writer is unable to suggest a more appropriate term. Moore (1933, p. 1160) states that, "With the data accumulated to date, it would appear that the difficulty of killing the 'resistant' red scale was due to the difficulty of reaching the insect through its scale (waxy covering) rather than to any distinct immunity of the insect to hydrocyanic acid."

The writer recognizes the waxy covering as an integral part of the insect itself. The red scale or any other armored scale insect would be no more likely to survive without its waxy covering than a turtle without its shell covering; at any rate, the shell covering is a part of the turtle. The turtle undoubtedly is more resistant to, say, a blow from a hammer, or to crushing, on account of the protection afforded by its hard covering.

Similarly, it may be granted that resistance to HCN in the red scale may be due to its covering.

Possibly the scale covering is not responsible[4]: the gas may get through or under the scale covering but be stopped at the entrance to the spiracles; or the gas may go through the spiracles and permeate the tracheal system, but the individual body cells show greater resistance to HCN. Whatever the mechanism may be, the fact remains that certain scale insects in certain localities are more difficult to kill by HCN than in certain other localities, and for want of a better designation, they are here called "resistant" scales.

Incidentally, it may be noted that two out of the three scale insects discussed herein, as having become resistant, have no waxy covering. If the scale covering accounted for the resistance in the red scale, then, at least, it would not make the problem easier to be obliged to find a different explanation in the case of the other two scales. Attention is also called to the fact that the scale insects in question are passive insects, fixed to the plant, and hence any such active behavior as is represented by the codling moth's entering an apple, as mentioned by Hough (1934, p. 551), is not involved.

In this paper no attempt is made to explain the mechanism of insect resistance to HCN. The question as to whether it is due to a difference in the waxy covering or whether this is more tightly sealed to the surface in the case of the red scale, to a difference in respiratory rate, to a difference in nervous response, or to some other factor or factors, is not investigated.

Resistance to Lime-Sulfur in San Jose Scale.—Melander (1914 and 1923) noted that unsatisfactory results were secured in a particular apple district in the State of Washington in controlling the San Jose scale, *Aspidiotus perniciosus* Comst., by spraying with lime-sulfur. He decided, finally, to apply identical solutions of lime-sulfur in a number of localities and to make examinations every two weeks of the scales killed. In his study 350,000 scales were individually examined.

Table 1 shows the results obtained at but three places: North Yakima, Sunnyside, and True's orchard, Clarkson, selected because the conditions for the experiment were ideal in each of these cases. These data show that in the Clarkson experiment from 4.0 to 17.0 per cent of the scales were alive six weeks after the application of the lime-sulfur sprays, as compared with 0.0 to 2.4 per cent in the other districts. In the case of

[4] Since the above was written, D. L. Lindgren has determined that the same difference in tolerance or resistance to HCN exists in the motile young of the red scale as in the adults. Since the motile young have no covering, this would appear to eliminate the covering as a factor in resistance.

sprays 7 and 8, which were oil sprays, there were no differences in the results in the different localities.

Although these figures are significant in suggesting a greater resistance of the San Jose scale in the Clarkson district, there are a number of variable factors present in such field experiments. Even precision-

TABLE 1

Effect of Spray Treatments on Mortality of San Jose Scale*

No.	Spray	Source of scales	Per cent of scales alive			
			When sprayed	After 2 weeks	After 4 weeks	After 6 weeks
1	Lime-sulfur, 5°	North Yakima	92	57	30	0.0
		Sunnyside	95	60	6	0.0
		Clarkson	95	90	77	8.0
2	Lime-sulfur, 3°	North Yakima	92	80	51	0.0
		Sunnyside	95	78	3	0.5
		Clarkson	95	92	81	13.0
3	Lime-sulfur, 2°	North Yakima	92	75	40	0.5
		Sunnyside	95	76	4	0.0
		Clarkson	95	90	76	17.0
4	Lime-sulfur, 1:½:5	North Yakima	92	88	35	0.1
		Sunnyside	95	93	2	0.2
		Clarkson	95	93	75	4.0
5	Lime-sulfur, 1:2:5	North Yakima	92	50	22	0.2
		Sunnyside	95	58	4	0.0
		Clarkson	95	77	52	8.0
6	Spraymulsion	North Yakima	92	44	2	2.0
		Sunnyside	95	50	7	2.4
		Clarkson	95	4	1	0.0
7	Orchard Brand spray	North Yakima	92	6	0	0.0
		Sunnyside	95	13	0	0.0
		Clarkson	95	4	1	0.0
8	Fuel oil emulsion	North Yakima	92	60	0	0.0
		Sunnyside	95	91	0	0.0
		Clarkson	95	62	0	0.0

* Source of data: Melander (1914).

spraying experiments as used in the modern laboratory leave some possibility of error. In the case of fumigation experiments, however, where two or more samples are in the same chamber and where all conditions are identical, these variable factors have been eliminated. Certainly, this should be the case where the different lots of scale are grown under identical conditions and on the same host before the fumigation, as has been done in the case of some of the experiments which will be presented.

Experiences with Resistance in the Red Scale.—In 1914, the writer's attention was called to unsatisfactory results from fumigation for the red scale, *Aonidiella aurantii* (Mask.), in the Corona district of California. The red scale was very abundant in a number of lemon groves there in spite of the fact that all the trees in a grove were fumigated in the fall, and the more severely infested ones again in the following spring. This condition was known to have prevailed for some years previous to 1914 and still prevails in 1937. In 1914, in practically all of the citrus area of California, the so-called "100 per cent" or "110 per cent schedule" (now 18- to 20-cc schedule) of dosage was so satisfactory that it was not necessary to repeat the fumigation for from two to four years. Such results also occur at the present time but not in the locality, or at least not in the grove, where the resistant scale exists.

The first suggestion about such unsatisfactory fumigation results was that either some details of the process had been overlooked or the dosage should be increased to insure better results. Attention to these factors, however, did not solve the difficulty. In experimental work, the dosage varied from 100 to 200 per cent (18 to 36 cc) for the regular period of 50 minutes, and dosages of from 75 to 100 per cent (14 to 18 cc)[5] repeated at the end of the regular period, failed to give satisfactory results. At this point the question was raised, "Are scales becoming resistant to hydrocyanic acid?"

The time and place and other variable factors may materially affect fumigation results. Hence, in any comparative work the variable factors must be eliminated. This was done by picking lemons infested with red scale from the different localities on the same day and keeping them under identical conditions both during and after the actual fumigation (Quayle, 1920).

Early in this work, red scale was found to be more likely to survive fumigation on lemon trees than on other varieties of citrus; on trees with heavy foliage, like the Lisbon lemon or the grapefruit, than on trees with sparse foliage; and on fruit than on foliage or branches. After the fruit, the scale is most difficult to kill on vigorous and thrifty leaves and suckers. The difference between various parts of the tree may be one of food supply. The difference between trees with heavy and sparse foliage may be accounted for by the greater sorptive capacity of the former.

All of these different field factors, however, while related to the general problem, bear only indirectly on the specific problem of red-scale resistance to hydrocyanic acid. The stages in the development of the red

[5] The term "100 per cent dosage" referred to sodium cyanide, 1 oz. to 100 cu. ft. for an average-sized tree, and "18 cc" refers to cubic centimeters of liquid HCN—a corresponding amount.

scale that are most tolerant to fumigation constitute a more closely related factor, and these stages have been determined as those beginning with and following the second molt.

First Observations of Resistance in the Black Scale.—In 1915, the writer's (Quayle, 1922) attention was called to the difficulty of killing the black scale, *Saissetia oleae* Bern., in the vicinity of Charter Oak, Los Angeles County. Prior to that time a 75 per cent dosage gave uniformly satisfactory results on the young black scale, such as would be present in that vicinity in the summer and early fall. Dosages greatly in excess of that ordinarily required to give 100 per cent control of the black scale failed in the Charter Oak district.

TABLE 2

SUMMARY OF LABORATORY EXPERIMENTS TO DETERMINE THE LOCALITIES THAT HAVE RESISTANT RED SCALE*

(Insects alive per 1,000 dead; 90 lemons representing each locality in both experiments)

Source of scales	Experiment 1			Experiment 2		
	Dosage, per cent			Dosage, per cent		
	100	125	150	85	125	150
Altadena	2	0	0	2	0	..
San Dimas	2	..	..	10	0	..
Corona	6	1	0	19	2	0
La Habra	24	5	0	15	..	0
Yorba Linda	7	1	0	30	3	2
Arlington	5	2	1	53	1	1
Orange	13	3	1	27	4	2
East Whittier	16	5	2	25	1	1

* From: Woglum (1925).

In 1925, Woglum published the results of his studies on the question of resistance in the red and black scales. His data on the red scale are included in table 2.

Concerning the black scale, Woglum (1925) states that his experimental results in 1907–1909 showed that this scale in its immature stages was effectively controlled throughout southern California with a 75 per cent schedule. His attention was directed to unsatisfactory fumigation results in the Charter Oak district, Los Angeles County, in 1912. Twelve years after resistance was first noted in the black scale, the resistant area had extended for a distance of 35 miles. He states, "Fumigation dosages of 100 to 120 per cent failed to give control for a second year, while previously a 75 per cent dosage usually gave control for two years."

Gray and Kirkpatrick (1929*b*) state, "We have determined by carefully planned experiments that resistance in the black scale is a reality."

First Observations of Resistance in the Citricola Scale.—Thus far two scale insects, namely, the red and the black scale, have been discussed as having developed a tolerance or resistance to hydrocyanic acid. A third scale insect, the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), developed a like resistance to fumigation (Quayle, 1932, p. 65). Resistance in this species was first observed in the Anthony orchard near Riverside, California, in 1925.

Value of Spray-Fumigation Treatment for Red Scale.—After twenty years of experimental evidence and field experience, which established the fact of greater resistance of certain scale insects in certain localities to hydrocyanic acid fumigation, Quayle and Ebeling (1934) published evidence to indicate that a spray-fumigation treatment was necessary to accomplish the most satisfactory results in controlling the resistant red scale on lemons.

Resistance to Lead Arsenate by Codling-Moth Larvae.—Hough (1928) conducted a series of experiments on the effect of lead arsenate on codling-moth larvae from Grand Valley, Colorado, and from the Shenandoah Valley, Virginia. He states:

> The Colorado larvae demonstrated a marked superiority over the Virginia larvae in their capacity to enter the sprayed apples. The strains were crossed, and the first generation of each cross was less resistant to arsenical poison than the pure strain of the Colorado larvae but more resistant than the Virginia larvae.

Hough in 1934 further states:

> The history of the codling moth in the Grand Valley of Colorado and the Shenandoah Valley of Virginia shows that the insect has been much more difficult to control in Colorado. The seasonal history of the codling moth is essentially alike in the two districts according to the published records.
>
> . . . .
>
> Colorado larvae reared under Virginia climatic conditions since 1928 have consistently demonstrated a distinct superiority over Virginia larvae in their ability to enter sprayed fruit.
>
> Greater ability of the Colorado larvae to enter sprayed fruit was not specific for lead arsenate but was also demonstrated when such nonarsenical sprays were used as cryolite, barium fluosilicate, rotenone, cuprous cyanide, and nicotine.
>
> . . . .
>
> This investigation demonstrates the existence of different strains of the codling moth in which the young larvae vary greatly in vigor. By rearing the larvae continuously on freshly sprayed fruit in the laboratory, it was possible to increase in a strain the proportion of individuals which possessed more vigor, with the result that a greater percentage of the young larvae entered and injured sprayed apples.

That an increased resistance or tolerance to certain insecticides has developed in "strains" or "races" of scale insects and the codling moth appears to be indicated from the published data already presented.

RESISTANCE IN THE CITRICOLA SCALE

The attention of the writer was first called to unsatisfactory fumigation results against the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), in a very limited area in the Riverside district in 1925. Prior to that date it was rather well established that an early fumigation, that is, before October 1, or still better, before September 15, resulted in a very satisfactory control of this scale. In fact, fumigators were so certain of the

Fig. 1.—Shaded areas show roughly the distribution of citrus in California south of the Tehachapi mountain range. *1*, The Riverside location where resistance was first noted (1925) in the citricola scale. Resistance later appeared in most of the areas occupied by the citricola scale, which are represented as crosshatched. *2*, Location of Highgrove. *3*, Location of Rialto, the places mentioned in tables 4 and 5.

results they could secure that in Tulare County the work was guaranteed, and if a satisfactory result was not secured, a second fumigation would be given without expense to the grower.

Fumigation experiments were conducted in the field at Riverside in nearly every month of the year (1926–27) to determine whether there might not be a season when the scale would be vulnerable. The dosage was greatly increased over that previously used, yet with unsatisfactory results. Some of the work was done in June, yet the recently hatched scales at that season were not killed with the highest dosage that could be used with safety to the trees.

The area where this tolerance to fumigation appeared extended very rapidly (fig. 1), and in the course of three or four years occupied much of the Riverside-Highgrove and Redlands-Cucamonga areas, or most of the area where the citricola scale occurred in southern California. This

resistance continued until the season of 1933–34, when, for some unknown reason, the citricola scale disappeared so completely through natural causes that, except in a few instances, treatment has not been renewed for this pest.

Much evidence for resistance in the citricola scale has been found in field experience. This experience has been so marked and clear cut that everyone acquainted with the facts has taken it as a matter of course. The very practical proof of resistance was the fact that fumigation, the recognized standard of control, was gradually replaced by spraying where resistance occurred. While the phenomenon of resistance was spreading rapidly, and the area was not clearly delineated, it was customary for fumigators to make tests on two or three trees to determine whether the scale in a particular planting would succumb to fumigation. If not, as was so frequently the case, spraying would be substituted.

Extensive laboratory tests have been made on the question of resistance of the citricola scale to cyanide fumigation.[6] The first series of comparative tests was conducted from March, 1928, to February, 1929. In these tests, orange twigs infested with citricola scale were cut from trees in orchards where commercial fumigation experience indicated that the scales were resistant and from other orchards where they were considered to be nonresistant. It should be noted that at this season of the year (December to February), commercial fumigation for the citricola scale is not practiced because the insects are not in a stage of development that is very susceptible to fumigation. For the purpose of comparative results, however, this season is satisfactory, and while high percentages in mortality are not secured, the differences between localities are strikingly shown.

Heavily infested twigs, from 4 to 6 inches in length, with 4 to 10 leaves, were planted separately in wet sand in 3-inch flower pots, fumigated in a fumatorium, and then kept for approximately three weeks under glass. The cuttings were watered daily in order to keep the soil and atmosphere practically saturated. Under these conditions the cuttings were kept in a healthy condition and the natural mortality of the scale, which was slight, was deducted. Thus the figures on the fumigation results give the net kill (table 3).

At the time the data given in table 3 were secured, there had been no evidence that the citricola scale in the orchards designated Highgrove, North Riverside, and Rialto did not yield satisfactorily to fumigation; hence the scales were considered as nonresistant or normal.

---

[6] The tests were made by A. F. Swain, of the Pacific R. and H. Chemical Corporation, with the coöperation of the writer in some cases.

TABLE 3

SUMMARY OF FUMIGATION EXPERIMENTS ON RESISTANT AND NONRESISTANT CITRICOLA SCALE, DECEMBER, 1928 TO FEBRUARY, 1929*

Experiment No.	Temperature, ° F	Relative humidity, per cent	HCN concentration, per cent	Source and type of scale	Per cent kill	Difference,† per cent kill
1	..	..	0.06	Highgrove, nonresistant......	76.9	....
				Arlington Heights, resistant..	66.3	10.6±8.4
2	63	51	0.07	North Riverside, nonresistant	70.4	....
				Arlington Heights, resistant..	28.4	42.0±7.6
3	63	47	0.08	North Riverside, nonresistant	81.2	....
				Arlington Heights, resistant..	27.9	53.3±7.4
4	67	30	0.10	Highgrove, nonresistant......	72.1	....
				Hermosa Rancho, resistant...	40.2	31.9±2.7
5	73	25	0.10	Highgrove, nonresistant......	79.2	....
				Hermosa Rancho, resistant...	58.7	20.5±5.5
6	56	71	0.10	Highgrove, nonresistant......	78.1	....
				Hermosa Rancho, resistant...	49.7	28.4±2.9
7	60	68	0.10‡	Highgrove, nonresistant......	59.5	....
				Hermosa Rancho, resistant...	35.4	24.1±6.7
8	54	76	0.10	Rialto, nonresistant..........	82.2	....
				Hermosa Rancho, resistant...	36.1	46.1±7.4
				Pomona, resistant............	30.0	52.2±4.7
9	53	70	0.10	Rialto, nonresistant..........	85.0	....
				Hermosa Rancho, resistant...	35.0	50.0±6.2
				Pomona, resistant............	28.1	56.9±4.5
A	60	31	0.08¶	Highgrove, nonresistant......	88.4	....
				Arlington Heights, resistant..	10.0	78.4±2.4
B	64	31	0.10¶	Highgrove, nonresistant......	94.4	....
				Arlington Heights, resistant..	13.3	81.1±4.5
C	51	45	0.10¶	Highgrove, nonresistant......	49.4	....
				Arlington Heights, resistant..	2.7	46.7±5.6
D	52	46	0.10¶	Highgrove, nonresistant......	56.3	....
				Arlington Heights, resistant..	9.2	47.1±4.0
E	57	27	0.20¶	Highgrove, nonresistant......	96.9	....
				Arlington Heights, resistant..	39.3	57.6±3.7
F	59	21	0.20¶	Highgrove, nonresistant......	93.7	....
				Arlington Heights, resistant..	47.9	45.8±1.9
Average	..	..	....	Nonresistant.................	77.6	....
				Resistant.....................	32.7	44.9

* From tests made by A. F. Swain; data used with his permission.

† Probable error of the mean employed in all tables.

‡ 0.10 per cent HCN plus 0.05 per cent methyl acetate.

¶ Experiments A to F used 4, 5, and 10 cc HCN in 100 cu. ft. fumatorium at Riverside. Percentage concentration only approximate.

The resistant scales were secured from:

1. A Valencia grove (lot 3, block 63, Arlington Heights, Riverside), which was fumigated in August, 1925, August, 1927, and September, 1928. When the scales were secured (December, 1928, to February, 1929), this was one of the most severely infested groves in the district.

TABLE 4

SUMMARY OF A SERIES OF TESTS ON THE COMPARATIVE RESISTANCE OF THE CITRICOLA SCALE TO HCN FUMIGATION*

Date	Number of tests	Source and type of scale	Per cent kill
Dec., 1928	2	Riverside, nonresistant Arlington Heights, resistant	57.1±3.8 29.5±0.8
Dec., 1929	7	Highgrove, nonresistant Arlington Heights, resistant	78.4±5.1 28.8±5.5
Jan., 1930	4	Highgrove, nonresistant Hermosa Rancho, resistant	72.8±2.8 44.6±3.7
Feb., 1930	2	Rialto, nonresistant Hermosa Rancho, resistant	83.7±0.9 37.2±0.6
All winter tests	15	Nonresistant Resistant	77.8±1.8 32.5±1.7
May, June, 1930	5	East Riverside, nonresistant Arlington Heights, resistant	94.5±2.6 68.0±9.8
July, 1932	6	Rialto, nonresistant Arlington Heights, resistant	99.5±0.2 23.2±3.4
July, 1932	6	Highgrove, nonresistant Arlington Heights, resistant	97.3±1.6 36.2±8.6
July, 1932	2	Highgrove, nonresistant Crafton, resistant	100.0 67.2±5.4
All summer tests	19	Nonresistant Resistant	98.9±0.5 42.2±3.6
Average all tests	34	Nonresistant Resistant	90.4±1.1 47.6±3.5

* From tests made by A. F. Swain; data used with his permission.

2. A grove (block 12, Hermosa Rancho, Riverside) not more than 3 miles distant from the grove mentioned above, which was fumigated in August, 1924, August, 1926, and July, 1928, and was heavily infested in December, 1928, to February, 1929, when the tests in table 3 were made.

3. A grove located between Pomona and Claremont, in Los Angeles County, which was chosen because of the failure to obtain satisfactory control of the citricola scale by fumigation on August 4, 1928.

The difference in survival, as indicated in table 3, was 44.9 per cent greater in the scale from the resistant groves than from those in the nonresistant groves.

Swain conducted another series of experiments from December, 1928, to July, 1932, with orange cuttings infested with citricola scale as before (table 4).

The groves recorded above, where the scale was normal or nonresistant, became distinctly resistant a few years later, so that by 1933 practically all of the groves in the general districts represented were classed as resistant, and fumigation was no longer generally recommended as a satisfactory treatment.

TABLE 5

Experiments to Determine the Dosage of HCN Necessary to Effect a Complete Mortality of the Resistant Citricola Scale*

(Exposure, 40 min.)

Date, 1929	HCN concentration, per cent	Result on scale	Plant condition
July 25	0.28	All alive	No injury
July 25	0.19	All alive	No injury
July 25	0.10	All alive	No injury
July 26	0.34	Few alive	Severe burn
July 26	0.25	Few alive	Slight burn
July 26	0.17	Few alive	Slight burn
August 4	0.48	All dead	Severe burn
August 4	0.40	Few alive	Severe burn
August 4	0.23	Few alive	Moderate burn
August 7	0.47	Few alive	Severe burn
August 7	0.40	Few alive	Severe burn
August 7	0.30	Many alive	Moderate burn
August 7	0.22	Many alive	Slight burn
August 7	0.11	Many alive	No injury

* From tests made by A. F. Swain; data used with his permission.

Further experiments carried on by Swain consisted of collecting twigs from orange trees infested with citricola scale in January, 1929, and allowing the scales from these infested twigs to migrate and to settle on one-year-old orange seedlings about 12 inches high, which were growing in 6-inch pots. Advantage was taken of the fact that the citricola scales that may begin their development on the leaves, migrate to the twigs in the winter and early spring. Furthermore, those scales that migrate to the twigs will leave such twigs if the twigs are allowed to dry. By July and August of the same year the seedling oranges became infested with young scale which represented the progeny of the scales that had been transferred to the seedlings as indicated.

The young scales thus established on the seedlings were fumigated in

July and August in a laboratory fumatorium. Their parents had come from both resistant stock (Hermosa Rancho) and from nonresistant or normal stock (Highgrove).

In the first experiment an attempt was made to determine the dosage and exposure necessary to obtain a 100 per cent kill in the resistant material only, as indicated in table 5. This table shows that in but one experiment all of the scales were killed. That was where the concentration of HCN was 0.48 per cent, or more than four times the dosage that

TABLE 6

EFFECT OF DIFFERENT DOSAGES AND EXPOSURES OF HCN ON THE RESISTANT AND NONRESISTANT CITRICOLA SCALE, AUGUST 13, 1929*

Source and type of scale	HCN concentration, per cent	Exposure, min.	Result on scale	Plant condition
Highgrove, nonresistant	0.05	40	Many alive	No injury
		60	All dead	No injury
		90	All dead	No injury
Hermosa Rancho, resistant	0.30	40	Many alive	No injury
		60	Many alive	Moderate burn
		90	Two alive	Severe burn

* From tests made by A. F. Swain; data used with his permission.

may ordinarily be used on citrus trees, and at a season for the best fumigation results.

A second experiment, also made by Swain, was similar to the first except that both resistant and nonresistant scales were treated with different dosages and exposures (table 6).

Table 6 shows a most striking difference between the dosages and exposures necessary to kill the resistant as compared with the nonresistant citricola scale. A concentration of 0.05 per cent HCN for 60 minutes was sufficient to effect a complete kill of the scale at Highgrove, whereas a concentration of 0.30 per cent HCN for 90 minutes was not sufficient to kill all of the scale at Hermosa Rancho. In other words, to effect a complete kill of the resistant scale required the combination of more than six times the dosage and one and a half times the exposure necessary for the nonresistant citricola scale in this experiment. Do not such results as given in tables 4, 5, and 6 present some evidence supporting the claim that there is a difference in tolerance, or resistance, of the citricola scale to HCN in some groves as compared with that in other groves?

As mentioned elsewhere, the citricola scale in a single season, from October, 1933, to the spring of 1934, disappeared so completely that treatment was discontinued. Up to this time, or from 1925 to 1933, re-

sistance continued, and the area came to include practically all groves in which the citricola scale occurred in southern California, including all of those given in the foregoing discussion. This scale began to reappear in a very few groves in 1936 and is expected to recur generally in the future. At such time, note will be made as to whether the resistance to HCN fumigation also recurs or whether it has disappeared and fumigation again becomes a generally satisfactory treatment as was the case from 1913 to 1925. Test fumigations made in the summer of 1937 indicate that resistance to HCN still continues.

RESISTANCE IN THE BLACK SCALE

Resistance to HCN fumigation in the black scale, *Saissetia oleae* Bern., has been determined mostly from field observation. Some of these observations have been referred to in a review of the literature, and some

TABLE 7

Difference in Response of the Resistant and Nonresistant Black Scale to HCN Fumigation*

Experiment No.	HCN concentration, per cent	Source and type of scale	Number of scales treated	Per cent kill
1	0.069 to 0.075	San Fernando, nonresistant	160	99.4
		San Dimas, resistant	957	90.6
2	0.067 to 0.073	San Fernando, nonresistant	96	100.0
		San Dimas, resistant	135	79.3

* Source of data: Gray and Kirkpatrick (1929*b*).

carefully planned experiments conducted by Gray and Kirkpatrick (1929 *a* and *b*) were mentioned. These experiments will now be briefly described:

One lot of sour-orange seedlings was infested with black-scale crawlers (motile young) from San Dimas, where the black scale was reputed to be resistant, and the other lot similarly infested with scales from San Fernando and North Whittier Heights, where fumigation still proved to be satisfactory. Four plants, two infested with scales from each locality, were placed in a gas-tight metal fumatorium and fumigated with the same dosage of HCN and the same exposure (40 minutes). Thus, three sets of four plants each were fumigated during the day, and three sets during the night. Table 7 gives a summary of the results.

Another experiment by Gray and Kirkpatrick consisted in subjecting 20 crawlers from each locality to a mixture of HCN and air of known

concentration. This experiment was repeated three times, the concentration being doubled each time. The results are shown in table 8.

TABLE 8

DIFFERENCE IN RESPONSE OF THE RESISTANT AND NONRESISTANT BLACK SCALE TO LOW, MEDIUM, AND HIGH CONCENTRATIONS OF HCN*

Source of scale	Per cent kill of scale at various concentrations of HCN		
	Low concentration	Medium concentration	High concentration
San Fernando, nonresistant	100	100	100
San Dimas, resistant	75	95	100

* Source of data: Gray and Kirkpatrick (1929*b*).

Resistance to fumigation of the black scale was first noted in the vicinity of Charter Oak in Los Angeles County about twenty-five years ago. By 1925 the area where this resistance occurred extended over a solidly planted citrus belt for a distance of about 40 miles, or most of eastern

Fig. 2.—Shaded areas show roughly the distribution of citrus in California south of the Tehachapi mountain range. *1*, The location of Charter Oak where resistance was first noted (1912–14) in the black scale. The area at present recognized as resistant includes roughly that which is crosshatched. The black scale occurs generally over all of the citrus area indicated except that in Imperial County and the area farthest east (Coachella Valley) in Riverside County.

Los Angeles and western San Bernardino counties. If the question of black-scale resistance in this area is doubted, all that is necessary is to try to control the insect by general fumigation. Here again the facts in the situation are shown by the commercial practice that has been in operation in the area for the past twenty-five years. It is here where oil sprays first came to be widely substituted for fumigation. This was partly be-

cause of the improvement in the type of oil spray used and the equipment for applying it, but much more because of the failure of fumigation to control the black scale. This was the period from about 1923 to 1928.

Outside of the general area mentioned, the black scale yields readily to fumigation dosages much smaller than those that fail to give control in the resistant area. In fact, the best proof supporting the question of resistance is the fact that only a rather small part of the citrus area exhibits this phenomenon (fig. 2), while the rest provides comparisons and checks. If, over the entire citrus area of California, the scale insects in question had become harder to kill by fumigation now than previously, it would be difficult to offer specific or experimental proof.

RESISTANCE IN THE CALIFORNIA RED SCALE[7]

Some of the more important published data and conclusions on the question of resistance to HCN fumigation in the red scale, *Aonidiella aurantii* (Mask.), have been given in the introduction.

A. F. Swain in 1928 and 1931 carried on tests on the comparative resistance of the red scale to fumigation under form trees and in a fumatorium. Table 9 is a summary of these tests.

TABLE 9

COMPARATIVE RESISTANCE OF THE RED SCALE TO HCN FUMIGATION FROM SEVERAL LOCALITIES, 1928 TO 1931*

Method of fumigation	Number of tests	HCN dosage, cc	Exposure, min.	Source and type of scale	Per cent kill
Under form trees	47	14 to 24	40	East Whittier, nonresistant	98.3±0.7
				Corona, resistant	91.8±0.8
In 100-cu. ft. fumatorium	12	10 to 20	10	Glendora, nonresistant	98.5±0.8
				Corona, resistant	94.0±1.3
			20	San Fernando, nonresistant	99.6±0.1
				Orange, resistant	95.8±0.9

* From tests made by A. F. Swain; data used with his permission.

A. F. Kirkpatrick carried on extensive tests on the comparative resistance of the red scale in different localities to fumigation during the season of 1936. He has kindly allowed the writer the use of his data, which have been compiled in table 10.

The scales from Fallbrook were secured from the lemon grove of

[7] The material included here consists of unpublished data that have been secured chiefly by A. F. Kirkpatrick, of the American Cyanamid and Chemical Corporation; by A. F. Swain, of the Pacific R. and H. Chemical Corporation; and by the writer and his colleagues in the University of California Citrus Experiment Station.

TABLE 10

COMPARATIVE RESISTANCE OF THE RED SCALE FROM FALLBROOK, ESCONDIDO, MONTECITO, GLENDORA, AND CORONA, 1936*

Experiments conducted under tents			Experiments conducted in fumatorium		
HCN concentration, per cent	Source and type of scale	Net per cent kill	HCN concentration, per cent	Source and type of scale	Net per cent kill
0.0119	Fallbrook, nonresistant Glendora, resistant.....	99.87±0.07 61.30±0.74	0.0195	Fallbrook, nonresistant Glendora, resistant.....	100.00 68.50±0.70
.0203	Fallbrook, nonresistant Glendora, resistant.....	99.87±0.07 70.15±0.69	.0339	Fallbrook, nonresistant Glendora, resistant.....	100.00 66.85±0.71
.0520	Fallbrook, nonresistant Glendora, resistant.....	100.00 80.15±0.60	.0591	Fallbrook, nonresistant Glendora, resistant.....	100.00 75.10±0.65
.1093	Fallbrook, nonresistant Glendora, resistant.....	100.00 96.30±0.29	.1020	Fallbrook, nonresistant Glendora, resistant.....	100.00 89.85±0.46
.0119	Escondido, nonresistant Corona, resistant.......	99.40±0.60 68.95±0.70	.0195	Escondido, nonresistant Corona, resistant.......	99.10±0.14 73.90±0.66
.0203	Escondido, nonresistant Corona, resistant.......	99.70±0.08 76.15±0.64	.0339	Escondido, nonresistant Corona, resistant.......	99.90±0.05 81.25±0.59
.0520	Escondido, nonresistant Corona, resistant.......	100.00 91.90±0.41	.0591	Escondido, nonresistant Corona, resistant.......	100.00 90.60±0.44
.1020	Escondido, nonresistant Corona, resistant.......	100.00 99.05±0.15	.1020	Escondido, nonresistant Corona, resistant.......	100.00 98.10±0.21
.0075	Montecito, nonresistant Corona, resistant.......	96.80±0.27 64.65±0.72	.0095	Montecito, nonresistant Corona, resistant.......	96.95±0.26 55.10±0.75
.0149	Montecito, nonresistant Corona, resistant.......	99.35±0.12 61.85±0.73	.0120	Montecito, nonresistant Corona, resistant.......	98.10±0.21 57.40±0.75
.0202	Montecito, nonresistant Corona, resistant.......	99.35±0.12 73.20±0.67	.0160	Montecito, nonresistant Corona, resistant.......	98.95±0.18 58.60±0.74
.0272	Montecito, nonresistant Corona, resistant.......	99.95±0.03 82.10±0.59	.0236	Montecito, nonresistant Corona, resistant.......	99.75±0.08 72.25±0.68
.0513	Montecito, nonresistant Corona, resistant.......	99.85±0.06 91.45±0.42	.0476	Montecito, nonresistant Corona, resistant.......	99.40±0.12 80.70±0.60
.0816	Montecito, nonresistant Corona, resistant.......	99.95±0.03 98.10±0.21	.0684	Montecito, nonresistant Corona, resistant.......	99.90±0.05 95.15±0.32
0.0941	Montecito, nonresistant Corona, resistant.......	100.00 97.45±0.24	0.0959	Montecito, nonresistant Corona, resistant.......	99.95±0.03 96.15±0.29

* From tests made by A. F. Kirkpatrick; data used with his permission.

Charles T. Wetzler, in the town of Fallbrook. This grove was sprayed with oil in 1935 and not treated in 1936.

The scales from Glendora were secured from the Frank Brown Washington Navel orange grove on Sierra Madre Avenue. This grove was fumigated twice in 1935 and sprayed with oil early in the season of 1936.

The scales from Escondido were from the lemon grove of Oscar Ander-

Fig. 3.—Shaded areas represent roughly the distribution of citrus in California south of the Tehachapi mountain range. The numbers represent the location of the chief points referred to in the tables and text in connection with the red scale: *1*, Montecito; *2*, San Fernando; *3*, Glendora; *4*, East Whittier; *5*, Orange; *6*, Upland; *7*, Riverside; *8*, Prenda; *9*, Corona; *10*, Fallbrook; *11*, Escondido. The red scale is more or less generally distributed in all the areas represented except that of Imperial County and eastern Riverside County (Coachella Valley). Resistance of the red scale to fumigation is recognized only in parts of the areas that are crosshatched.

son, located 4 miles east of Escondido. This grove was oil-sprayed and fumigated during the 1934 season. It was not treated in 1935 or 1936.

The scales from Corona were from the lemon grove of Miss Sarah Thrasher. This grove was fumigated with a heavy dosage 11 weeks before the material used in the experiments was collected.

The scales from Montecito were from the Crocker-Sperry lemon grove. The grove had not been treated for scale control for two years previous to the collection of the material. Fumigation in the past had given satisfactory control.

The groves at Fallbrook, Escondido, and Montecito were selected as representing nonresistant red scale, while those at Glendora and Corona were selected as representing resistant red scale. A glance at the results in table 10 will show that here again laboratory tests verify field experience. Even with the highest dosages used, a satisfactory kill of the

resistant scale was not secured; whereas with much lower dosages a satisfactory kill of the nonresistant scale was obtained.

In connection with some further work on the relation of temperature to fumigation results on the red scale, D. L. Lindgren and the writer had occasion to use red scale from resistant and nonresistant groves. Some of the figures have been taken from that study (table 11) to show the difference in resistance of the red scale to fumigation. Three localities are represented. The scales from Prenda were on lemons from the F. G. Lewis grove; those from Glendora were on lemons from the Coulson

TABLE 11

COMPARATIVE RESISTANCE OF RED SCALE FROM PRENDA, CORONA, AND GLENDORA TO HCN FUMIGATION IN 100 CU. FT. FUMATORIUM

(Exposure, 40 min.)

Source of scales	HCN		Number of fumigations	Total number of scales	Net per cent kill
	Dosage, cc	Concentration, per cent			
Prenda (resistant)..........	3	0.0499	18	36,817	92.36±0.09
		.0526	14	28,447	92.76±0.10
		0.0510	15	31,254	92.00±0.10
Corona (resistant)..........	3	0.0445	9	18,242	81.00±0.18
Glendora (nonresistant)....	2	0.0314	9	16,970	99.60±0.03
		.0312	9	14,963	99.20±0.04
		0.0334	9	15,355	98.40±0.06

grove; and those from Corona were on lemons from the Tetley grove. The Glendora scales will be seen to be the most susceptible to HCN, and those from Corona, the most resistant. The scales from Prenda occupy a place between the two. The Prenda scales from field experience are in the resistant class, but they are not so resistant as those from particular places in the Corona district.

According to table 10, the Glendora scales were among the most resistant that were employed in that experiment. This fact is readily accounted for, and again it accords with field experience. The phenomenon of resistance in the Glendora area is much more recent (past four or five years) than in Corona and in some of the other areas. Consequently, resistance in the Glendora area is recognized as spotted among different groves and more prevalent in the higher foothill areas. The Brown grove, represented in table 10, is in an area well known to be a resistant area. On the other hand, in the particular block of the Coulson grove in table 11, not more than 3 miles away, the scales are nonresistant. In fact, there

may be different degrees of resistance in the red scale in different groves within the same locality. There is not at present a sharp division between resistant groves or areas and nonresistant groves or areas. The area where the increase in resistance occurs has been continuously enlarging for the past twenty-five years. Twenty-five years ago it was limited, so far as then known, to a single locality. Now there are many localities and numerous groves (fig. 3).

In table 11, where there was another object in view in securing the data, the resistant and nonresistant scales were not fumigated at the same time, as was the case with experiments thus far reported. They were

TABLE 12

Comparative Resistance of the Red Scale in West Riverside and Corona to HCN Fumigation

(Exposure, 40 min.)

HCN		Source of scales	Total scales	Per cent kill
Dosage, cc	Average concentration, per cent			
3	0.0496	West Riverside (nonresistant)	36,162	99.2±0.09
		Corona (resistant)	27,123	83.3±0.20
14*	0.0523	West Riverside (nonresistant)	33,921	97.5±0.07
		Corona (resistant)	28,367	91.3±0.15

* Gas gradually withdrawn to reduce average concentration.

fumigated in the same enclosure (100 cu. ft. fumatorium), and immediately after one another. The difference between the two groups—the Glendora scale (nonresistant) and the Prenda and Corona scale (resistant)—is very great in spite of the difference in dosage. Scales from Corona and Prenda were not completely killed with a 3-cc dosage. Even with one-third reduction in the dosage, or 2 cc, there was a higher percentage killed in the case of the Glendora scales.

Table 12 shows a comparison of red scale from Corona and West Riverside. In these tests 0.5 cc of HCN for 5 minutes was given as a stupefying charge[8] and then followed, in the first experiment, with 3 cc, the gas being maintained at this dosage throughout the exposure; in the second experiment, a 14-cc charge followed, but the gas was gradually withdrawn to make about the same mean concentration in both experiments. What is important here, however, is that the scales from the two localities were

[8] The meaning of the stupefying charge is fully discussed in the second article of this number of *Hilgardia:* Lindgren, D. L. The stupefaction of red scale, *Aonidiella aurantii,* by hydrocyanic acid. Hilgardia 11(5):211–25. 1938.

fumigated under identical conditions and that again a difference in kill, as between two different localities, is shown.

In another series of experiments (table 13), similar to those given in table 11, the dosage used against the nonresistant scale was one-fifth less than that used against the resistant scale, yet the percentage of kill was much higher in the former.

Four years ago the writer started colonies of the resistant and nonresistant red scale in separate insect-proof rooms in the insectary. The

TABLE 13

Comparative Resistance of the Red Scale from Corona and Glendora to HCN Fumigation in a 100 Cu. Ft. Fumatorium

(Exposure, 40 min.)

Source and type of scales	HCN		Number of fumigations	Total number of scales	Net per cent kill
	Dosage, cc	Concentration, per cent			
Corona, resistant	2.5	0.0447	16	35,734	84.2±0.18
Glendora, nonresistant	2.0	0.0317	14	33,521	98.7±0.05

scales were transferred from lemons to seedling orange trees. A test fumigation of these scales the following year showed that the difference in resistance to HCN was maintained. Conditions brought about in other parts of the insectary that were suitable for other work proved later to be adverse to the colonies of red scale, which were practically all destroyed. Moreover, small seedling citrus trees proved unsatisfactory for maintaining the colonies. Some of the trees would become so badly infested that the tree would be killed, and sometimes the results of transferring the scales were not very successful. Moreover, there were no fruits present under these conditions, whereas all the experimental work done heretofore was with scales on the fruits.

In the summer of 1936 new colonies of resistant and nonresistant red scale were started by D. L. Lindgren and the writer; these were kept in separate insect-proof rooms on banana squash, a host that was originally found by Rush Bumgardner, of the Orange County Insectary, to be suitable for the rearing of red scale. These colonies were obtained from the Tetley lemon grove at Corona and from a block in the Coulson groves at Glendora. The differences in tolerance to HCN of these original colonies is shown in tables 11 and 13.

These colonies of resistant and nonresistant scales are to be maintained, according to present plans, for a period of at least four years. Some of the scales will be fumigated from time to time during this period

TABLE 14

DIFFERENCE IN FUMIGATION RESULTS ON RESISTANT AND NONRESISTANT RED SCALE GROWN ON BANANA SQUASH

(Exposure, 40 min.)

Date	HCN		Temperature, °F	Humidity, per cent	Source and type of scale	Total number of scales	Net per cent kill
	Dosage, cc	Concentration, per cent					
Original stock on lemons							
Aug. 25, 1936	3	0.0512	80	70	Glendora, nonresistant...	1,606	100.00
					Corona, resistant........	974	91.70
Progeny on squash							
Nov. 24, 1936	3	0.0437	75	40	Glendora, nonresistant...	490	99.80
					Corona, resistant........	299	89.60
Nov. 28, 1936	2	.0391	75	40	Glendora, nonresistant...	305	99.34
					Corona, resistant........	410	68.80
Dec. 8, 1936	3	.0524	75	30	Glendora, nonresistant...	150	100.00
					Corona, resistant........	75	91.70
Dec. 18, 1936	3	.0465	73	30	Glendora, nonresistant...	878	99.98
					Corona, resistant........	924	93.32
Jan. 4, 1937	3	.0482	75	40	Glendora, nonresistant...	1,255	100.00
					Corona, resistant........	1,101	93.01
Jan. 13, 1937	2	.0346	75	35	Glendora, nonresistant...	1,229	95.68
					Corona, resistant........	1,652	84.10
Jan. 25, 1937	12*	.0511	75	40	Glendora, nonresistant...	196	100.00
					Corona, resistant........	304	98.98
Feb. 25, 1937	12	.0378	75	50	Glendora, nonresistant...	925	100.00
					Corona, resistant........	580	89.30
Feb. 25, 1937	2	.0391	75	50	Glendora, nonresistant...	825	99.87
					Corona, resistant........	410	96.83†
Mar. 27, 1937	3	.0472	75	48	Glendora, nonresistant...	800	100.00
					Corona, resistant........	849	96.41
May 3, 1937	2	0.0351	75	45	Glendora, nonresistant...	600	99.34
					Corona, resistant........	432	92.83

* 12-cc charge given, then exhaust operated to reduce mean average concentration as given.
† Squash in bad condition.

while others will be fumigated after one, two, three, and four years. The object is to determine whether resistance is maintained through a period of years while the resistant and nonresistant insects are grown under identical conditions; the effect of fumigation at intervals, as compared with a lapse of fumigation for as much as four years, will also be studied.

Experiments are also under way to determine, by crossing resistant females with nonresistant males and vice versa, whether these two strains of scales may be different species. If breeding occurs, the F_1 progeny will be fumigated to determine whether resistance is transmitted in these crosses. Mass breeding is at present under way, but colonies will later be obtained from individual pairs.

The results of fumigation up to the present time on the resistant and nonresistant red scale grown on banana squash under identical conditions in separate insect-proof rooms in the insectary are given in table 14. This table shows that up to May 3, 1937, nine months after the scales were originally transferred to squash and had gone through four generations, difference in resistance in the two colonies was maintained. How long the difference will continue remains to be determined.

Thus far data have been presented on the difference in resistance of three different scales from different localities to HCN fumigation. It is hoped that sufficient data have been presented to enable readers to arrive at a conclusion regarding the phenomenon of resistance to the fumigant in the three scale insects mentioned. There remains to be considered the difference, if any, in the resistance of some of the same insects to other fumigants.

DIFFERENCES IN THE RESISTANCE OF THE RED SCALE FROM DIFFERENT LOCALITIES TO FUMIGANTS OTHER THAN HCN

While a difference in tolerance of the red scale from different localities to HCN has been taken as a matter of course for the past twenty years, it was somewhat of a surprise to find that apparently the same difference in tolerance is manifested toward at least some other fumigants. Data with respect to other fumigants must be considered as only preliminary, but experiments to date show that they are significant.

Thus far but three fumigants other than HCN have been tried. One of these, carbon disulfide, is not included because the effect of this fumigant on the fruit, with the dosages used, was such as to make the results unreliable. The other two fumigants were methyl bromide and ethylene oxide.

The results with these fumigants are given in tables 15 and 16. These data seem to indicate that there is such a phenomenon as resistance in the red scale to two additional fumigants, namely, methyl bromide and ethylene oxide. Resistance to still other fumigants is now suspected to exist.

Several years ago, Knight, at that time a colleague of the writer, concluded that the resistant red scale was more resistant to oil sprays than

TABLE 15

Difference in Tolerance of Red Scale from Glendora and Corona to Methyl Bromide, in 100 Cu. Ft. Fumatorium

(Exposure, 45 min.)

Date, 1937	Dosage, ounces	Temperature, °F	Humidity, per cent	Source and type of scale	Total scales	Net per cent kill
Jan. 8	8	75	35	Glendora, nonresistant...	1,508	99.40
				Corona, resistant.........	1,356	28.10
Jan. 8	10	75	35	Glendora, nonresistant...	1,596	95.30
				Corona, resistant.........	1,627	77.40
Jan. 11	8	75	35	Glendora, nonresistant...	1,787	66.80
				Corona, resistant.........	1,827	38.10

TABLE 16

Difference in Tolerance of Red Scale from Glendora and Corona to Ethylene Oxide in 100 Cu. Ft. Fumatorium

(Exposure, 45 min.)

Date, 1937	Dosage, ounces	Temperature, °F	Humidity, per cent	Source and type of scale	Total scales	Net per cent kill
Jan. 7	6	75	35	Glendora, nonresistant...	1,322	98.70*
				Corona, resistant.........	1,783	92.80*
Jan. 9	4	75	35	Glendora, nonresistant...	1,672	42.60
				Corona, resistant.........	722	14.36
Jan. 9	6	75	35	Glendora, nonresistant	1,626	87.20
				Corona, resistant	1,704	67.90

* The lemons were injured somewhat in this experiment, which may account for the higher percentage of scales killed than in the third experiment where the same dosage was used.

the nonresistant scale; and Chamberlin, another colleague, engaged in the same investigation, concluded that there was a difference between the resistant and nonresistant red scale with reference to desiccation. The writer considered that the data supporting these claims were insufficient, and hence statements to that effect did not appear in the final manuscript (deOng, Knight, and Chamberlin, 1927). If the red scales from different localities show a difference in tolerance to fumigants in

general, it is possible that the resistant red scale is a hardier strain and may show a greater tolerance to sprays and to adverse conditions generally. It will be recalled that Hough (1934) in Virginia found the Colorado strain of codling moth to be resistant to all sprays tried and a hardier strain in general.

DISCUSSION

The greater tolerance or resistance to HCN fumigation that has developed in three of the most important citrus scale insects in certain localities in California has been brought about, apparently, through natural selection (Dobzhansky, 1937). The areas where the phenomenon first became evident are not necessarily the areas where fumigation has been practiced the longest. It is possible, however, that the areas where resistance first appeared had received as many fumigations as any other area, perhaps even more: that is, fumigations may have been more frequent over a given period. The question of elevation brings in other factors. On account of the warmer winter temperatures on the higher elevations, and also the higher night temperatures in summer, the red scale develops more rapidly under such conditions. Under such conditions, also, the lemon is extensively grown; and it is fairly well established that of the citrus varieties, the lemon is a favorite host. Of the different parts of the tree, the fruit is the one on which the red scale is most difficult to kill. In the case of the lemon, there are generally fruits, of some size, on the tree throughout the year. All of these factors, aside from any question of resistance, make the control of the red scale on the lemon more difficult. Nevertheless, when red-scale-infested lemons are taken from such situations and fumigated under identical conditions with other red-scale-infested lemons from other localities, the difference in tolerance or resistance is still manifest. And when the scales are transferred to another host and are grown under identical conditions, this difference still exists.

The phenomenon of resistance probably appeared in the first instance through mutations, or because of the presence of a mixture of the resistant and nonresistant strains in the original infestations in the California orchards. Which of these explanations is correct will probably remain forever obscure, in the opinion of Dobzhansky (1937, p. 161).

The history of the development of resistance in the red and black scales gives some support to the theory that such resistance spread from an original focus. In case of the red scale, it was first observed in the vicinity of Corona, in Riverside County, and at about the same time near Orange, in Orange County. It gradually spread in these areas and con-

tinued to La Habra, Orange County; East Whittier and North Whittier Heights, in Los Angeles County; and finally to Glendora and points to the east also in Los Angeles County. This, in general, is the higher foothill lemon area.

Resistance in the black scale first came to our notice in the vicinity of Charter Oak, in Los Angeles County. The area spread to the west, and to the east as far as the western part of San Bernardino County. This is almost a contiguous area as contrasted with the more or less spotted areas where the resistant red scale occurs.

The resistant citricola scale was first observed in the vicinity of Riverside, in Riverside County, but in the course of a few years it occurred over practically all of the citricola-scale areas, some of which are rather widely separated by barren land. It scarcely seems possible that an insect or strain could spread over such an extensive area in that length of time, especially where the infested areas were not contiguous.

The writer believes that for the most part the phenomenon of resistance in the three insects discussed has developed on the ground, and while some immediate spread would be expected, this factor has not accounted for the present distribution as having come from an original strain in a single locality.

The fumigation dosages now used in California even in the nonresistant areas, are much higher than those used many years ago. The schedules have been revised several times and always upward. It is interesting to note also that in Australia, South Africa, and Palestine, countries much younger than California in fumigation practice, the dosage used against the same insect is much lower than in California.

SUMMARY

It has been shown that three of the important citrus scales in California, the red scale, *Aonidiella aurantii* (Mask.); the black scale, *Saissetia oleae* Bern.; and the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), from certain localities in California have exhibited a greater tolerance or resistance to hydrocyanic acid fumigation than the same insects from other localities.

In the case of the red and black scales, the phenomenon of resistance has been noted since 1912 or 1914 and still exists.

Resistance in the citricola scale has been recognized since 1925. Considering the length of time fumigation has been practiced with this species, resistance developed much more rapidly than in the case of the red and black scales. The citricola scale was first recognized as a distinct species in the citrus groves of California about 1908 or 1909.

Experiments reported herein indicate that at least the red scales from different localities also exhibit a difference in tolerance to fumigants other than HCN, namely, to methyl bromide and ethylene oxide. It is suspected that this same difference in resistance may be shown to other fumigants not yet tested.

The areas where resistance has developed in the red, black, and citricola scales represent only a small part of the total citrus area in California. Outside of these resistant areas, fumigation continues to be the most satisfactory treatment for control; and even in the case of the resistant red scale, fumigation is still the most satisfactory single treatment. The black and citricola scales, representing unarmored scales, are more easily controlled by spraying than the red; hence spraying is more generally employed in the areas where black and citricola scales are resistant.

ACKNOWLEDGMENTS

Valuable unpublished data bearing on the subject of this paper have been kindly furnished by A. F. Swain, of the Pacific R. and H. Chemical Corporation, and by A. F. Kirkpatrick, of the American Cyanamid and Chemical Corporation. The writer gratefully acknowledges the addition of these data, and the assistance of his colleague, D. L. Lindgren.

LITERATURE CITED

Cunningham, G. H.

1935. Plant protection by the aid of therapeutants. 243 p. John McIndoe Pub., Dunedin, New Zealand.

DeOng, E. R., Hugh Knight, and J. C. Chamberlin.

1927. A preliminary study of petroleum oil as an insecticide for citrus trees. Hilgardia **2**(9):351–84.

Dobzhansky, Theodosius.

1937. Genetics and the origin of species. 364 p. Columbia Univ. Press, New York.

Gray, George P., and A. F. Kirkpatrick.

1929*a*. Resistant scale insect investigations. California Citrograph **14**(part I) 308, 336; (part II)364, 380–81.

1929*b*. Resistance of the black scale, *Saissetia oleae* Bern., to hydrocyanic acid fumigation. Jour. Econ. Ent. **22**:893–97.

Hough, W. S.

1928. Relative resistance to arsenical poisoning of two codling moth strains. Jour. Econ. Ent. **21**:325–29.

1929. Studies of the relative resistance to arsenical poisoning of different strains of codling moth larvae. Jour. Agr. Research **38**:245–56.

1934. Colorado and Virginia strains of codling moth in relation to their ability to enter sprayed and unsprayed apples. Jour. Agr. Research **48**:533–53.

Melander, A. L.

1914. Can insects become resistant to sprays? Jour. Econ. Ent. **7**:167–73.

1915. Varying susceptibility of the San Jose scale to sprays. Jour. Econ. Ent. **8**:475–81.

1923. Tolerance of San Jose scale to sprays. Washington Agr. Exp. Sta. Bul. (Technical Paper) **174**:1–52.

Moore, William.

1933. Studies of the "resistant" California red scale, *Aonidiella aurantii* Mask., in California. Jour. Econ. Ent. **26**:1140–61.

Quayle, H. J.

1916. Are scales becoming resistant to fumigation? California Univ. Jour. Agr. **3**:333–34, 358.

1920. Recent fumigation developments. California Citrograph **5**:188–89, 193.

1922. Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. Jour. Econ. Ent. **15**:400–404.

1932. Biology and control of citrus insects and mites. California Agr. Exp. Sta. Bul. **542**:1–87.

Quayle, H. J., and Walter Ebeling.

1934. Spray-fumigation treatment for resistant red scale on lemons. California Agr. Exp. Sta. Bul. **583**:1–22.

Woglum, R. S.

1925. Observations on insects developing immunity to insecticides. Jour. Econ. Ent. **18**:593–97.

THE STUPEFACTION OF RED SCALE, AONIDIELLA AURANTII, BY HYDROCYANIC ACID

D. L. LINDGREN

THE STUPEFACTION OF RED SCALE, AONIDIELLA AURANTII, BY HYDROCYANIC ACID[1, 2]

D. L. LINDGREN[3]

INTRODUCTION

Both the red scale, *Aonidiella aurantii* (Mask.), and the black scale, *Saissetia oleae* Bern., become more difficult to kill if they have first been exposed for a short time to a sublethal concentration of hydrocyanic acid gas. The term applied to the effect of small charges of HCN is "protective stupefaction." It may be brought about in the field by the leakage of gas through the tents or by poor diffusion of the gas within the tent. Since most workers agree that the red scale becomes stupefied when prefumigated with a sublethal concentration of HCN, an investigation was begun to determine the length of time these insects remain stupefied.

EARLIER INVESTIGATIONS

Gray and Kirkpatrick (1929) concluded that under the laboratory conditions of their experiments:

> Both the resistant and nonresistant strains of black and red scales exhibit a characteristic which is termed "protective stupefaction," that is, when a lot of scale is first exposed to a sublethal, but stupefying concentration of hydrocyanic acid in air, followed by a normally lethal concentration, more of them are able to survive than a lot upon which the reverse procedure has been followed.
>
> . . . .
>
> Correlated field and laboratory observations and experiments, not fully described in this paper, furnish good circumstantial evidence that protective stupefaction is sometimes a factor adversely affecting the results of scale kill in commercial fumigation.

Pratt, Swain, and Eldred (1931) found that protective stupefaction is a fact in the case of both black and red scales when exposed to lethal concentrations of HCN after 10- or 3-minute exposures to sublethal concentrations, but that protective stupefaction does not follow exposure for only 1 minute to sublethal concentrations.

---

[1] Received for publication February 14, 1938.

[2] Paper No. 347, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Junior Entomologist in the Experiment Station.

Moore (1933) states:

> Under conditions giving high kills prefumigation reduced the kill. Above 98 per cent there were four significant negative differences and two without differences. Below 98 per cent, the differences are all positive but only one is significant. These results indicate that exposure to low concentrations preceding the regular fumigation does not always reduce the kill and field results confirm these conclusions.

EXPERIMENTAL METHODS

A series of laboratory fumigation experiments was begun in 1935 on lemons infested with resistant red scale obtained from a single grove in the Corona district. In each test 18 to 20 lemons were used, and previous to fumigation they were held at 70° to 74° F for 18 to 60 hours. All experiments except those conducted in 1935 were carried on in pairs, that is, 18 to 20 lemons infested with red scale were subjected to a stupefying charge of 0.5 cc of HCN for 5 minutes in a 100 cu. ft. fumatorium. They were then removed and placed in a second fumatorium of the same size, along with 18 to 20 lemons which had not been exposed to a sublethal charge, and the two groups were subjected to the lethal charge for 40 minutes. The temperatures at which the fumigations were conducted ranged from 73° to 76° F and the relative humidity from 35 to 60 per cent. Within this humidity range, there seemed to be no effect on the fumigation results. Quayle and Rohrbaugh (1934) also found no significant differences in the effect of humidity within a range of from 50 to 80 per cent on fumigation results with red scale.

The lethal charges of HCN were administered in two different ways: one in which a low dosage—usually 3 cc but sometimes 2 or 4 cc—of HCN was added to the fumatorium and allowed to remain 40 minutes; and the other in which 12 cc of HCN was added and the exhaust pump was operated for the 40 minutes. The first produces a low, uniform concentration and the second a high-peak concentration similar to that obtained in commercial fumigation practice.

A sample was kept from each lot of fruit picked to determine the natural mortality of the red scale. About 1,000 mature female scales were examined on the untreated lemons at the same time that counts were made on the fumigated lots. The natural mortality varied from 48 to 57 per cent in the different untreated samples. In the tables the total number of scales indicated is the number of live scales present before treatment. Actually about twice as many red scales were examined in the tests, but since approximately one-half (48 to 57 per cent) of these represent the population which had died from natural causes, they were not included in the tables. In each case the percentage of natural mortality

was assumed to be the same in the treated sample as in the corresponding untreated one.

Only mature females were included in the examinations, which were made from 10 to 14 days after the fumigations. The experiments were numbered and dated only, and thus the several workers examining the scales had no knowledge of the treatment the insects had received.

The significance of the means was determined by the method outlined by Snedecor and Irwin (1933). This method takes into consideration the variance within the samples; and a comparison is made of the differences within groups as well as between groups to determine whether the means of the different methods of treatment are significant. A probability (P) of less than 0.05 is regarded as small enough to justify the conclusion that the differences are not due to random sampling.

PRELIMINARY TESTS WITHOUT A STUPEFYING CHARGE, COMPARING A LOW, UNIFORM CONCENTRATION WITH A HIGH-PEAK CONCENTRATION

Preliminary tests were made in 1935, to determine the mean concentration with the two methods of administering the dosage. The experiments also afford a comparison of the kill with the two types of concentration when used without a stupefying charge; and when compared with similar fumigations in subsequent experiments, they give an indication of the variation in results from year to year.

In the 1935 tests both the low, uniform concentration (with a 3-cc dosage) and the high-peak concentration (with a 12-cc dosage) were used (fig. 1). The mean average concentrations were calculated from the formula given by Knight (1925), $\frac{\Sigma\, MC \times T}{\Sigma\, T}$, where MC equals the mean concentration for each time interval over which MC is computed. As evaporation and diffusion were very rapid in the fumatorium (less than ½ minute), the mean average concentration was calculated from the time the cyanide was added. Samples of gas for titration were taken at 1, 4, 7, 15, 30, and 40 minutes from the time fumigation began. The mean average concentrations obtained from the two types of curves did not vary greatly, as can be seen from figure 1.

The 1935 experiments are summarized in table 1. These data indicate but slight difference in the kill with the two types of concentration.

In order to indicate the variation in results from year to year, those results of later experiments (see p. 217–218) that deal with scales fumigated without a stupefying charge are also summarized in table 1. The

red scales used in 1936 and 1937 were obtained from the same grove and in the same season as those used in the earlier experiments. In 1936 the low, uniform type of concentration was the only one tried. In 1937 this type was used with 9 lots, but most of the lots were fumigated with the high-peak type of concentration.

If results with the same type of concentration in different years are compared, they will be seen to vary rather widely from year to year.

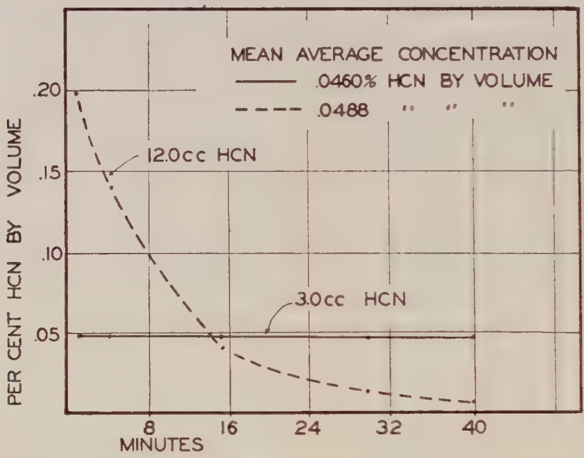

Fig. 1.—Types of concentration to which the red scales were exposed.

TABLE 1

FUMIGATION OF RESISTANT RED SCALE FROM 1935 TO 1937: A COMPARISON OF THE EFFECTS OF HIGH-PEAK (12 OR 14 CC) AND LOW, UNIFORM (3 CC) DOSAGES OF HCN

Dosage, cc	Time of fumigation	Number of experiments	Live scales treated	Number of scales killed	Per cent killed
3	Fall, 1935	16	14,699	13,382	91.04
14	Fall, 1935	15	12,408	11,463	92.38
3	Fall, 1936	37	34,398	28,806	83.74
3	Fall, 1937	9	5,280	4,582	86.78
12	Fall, 1937	47	41,724	36,972	88.61

Caution must therefore be exercised in comparing the different types of concentration in different years. In comparing the kill of red scale obtained by a low, uniform concentration in 1936 with that obtained by a high-peak concentration in 1937, for example, it must be remembered that, even though the red-scale-infested lemons were picked from the same grove, there was a lapse of one year between the time the two types of dosages were used. During this year, the scale had passed through

some severe and prolonged cold weather and one oil-spray treatment early in the spring of 1937. The difference in favor of the high-peak type of concentration is consequently of little or no significance.

RESULTS OBTAINED IN 1936 WITH A LOW, UNIFORM TYPE OF CONCENTRATION

The plan at the beginning of this problem was to determine the importance of protective stupefaction and the length of time red scale would remain stupefied after they had been exposed to a sublethal charge of HCN. Experiments were carried out in four groups: with no time interval and with 1 hour, 2 hours, and 3 hours between the stupefying charge and the regular fumigation. Work on all four groups of experiments was simultaneous, no one group being completed before another was started.

TABLE 2

FUMIGATION OF LEMONS INFESTED WITH RESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES IN 1936

(Stupefying charge, 0.5 cc of HCN; fumigating charge, 3 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of experiments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Probability, P
None	Fumigated	11	10,442	8,806	84.33	−10.36	<0.01
	Stupefied and fumigated	11	9,940	7,353	73.97		
1 hour	Fumigated	8	7,544	6,295	83.44	−7.12	0.03
	Stupefied and fumigated	8	8,077	6,165	76.32		
2 hours	Fumigated	9	8,458	7,093	83.86	+2.33	0.11
	Stupefied and fumigated	9	7,910	6,818	86.19		
3 hours	Fumigated	9	7,954	6,612	83.13	+4.81	0.04
	Stupefied and fumigated	9	7,926	6,970	87.94		

* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

In the no-time-interval experiments, fumigation with 3 cc HCN followed immediately after the stupefying charge. (See "Experimental Methods," p. 214, for description of procedure.) In the other groups of experiments, the procedure was the same except that the lemons were removed from the fumatorium and remained under atmospheric conditions for the interval designated. In each case a check lot of lemons infested with red scale that had not been exposed to the stupefying charge was fumigated at the same time. Each time interval was tested from 8 to 11 times.

Table 2 summarizes the results of these experiments. The red scale

exposed to a small dose of HCN 1 hour or less before fumigation survived the regular fumigation better than those not so exposed; but when exposed to the stupefying charge 2 or 3 hours before fumigation, they did not survive so well as those not exposed. The difference between mean percentage killed when a stupefying charge was given and that when none was given was — 10 per cent with no time interval, — 7 per cent with a 1-hour interval, + 2 per cent with a 2-hour interval, and + 5 per cent with a 3-hour interval. The difference is significant in each case except in that with the 2-hour interval, which is low enough to be due to chance variation. Apparently the resistant red scale succumbs to the stupefying charge immediately and remains stupefied for at least 1 hour.

The reversal of the difference in the 2-hour and 3-hour intervals may be the result of a higher rate of respiration of the insects after having been in a stupor for some time. Up to the present time, no other explanation can be offered. The close association of the red scale with its host makes the rate of respiration difficult to measure, and its waxy covering prevents any observation on its activity.

A few tests were conducted in which there was an interval of 4 hours between the sublethal and the lethal charges of HCN. In this series the difference in kill resulting from the two types of treatment was not significant, which indicates that at the end of 4 hours after receiving a stupefying charge, the resistant red scales react normally to the regular fumigation procedure.

RESULTS OBTAINED IN 1937 WITH THE HIGH-PEAK TYPE OF CONCENTRATION

On checking over the results of the experiments carried on in 1936, the question arose as to whether red scale after having been stupefied would react similarly to a high-peak type of concentration. It was thought that a high-peak concentration might overcome the effects of a stupefying charge to some extent. Therefore a series of experiments was conducted in 1937 along lines similar to those of 1936, with the exception that 12 cc of HCN were used in the fumatorium as the lethal charge and the gas was gradually withdrawn from the chamber to give the type of concentration curve shown in figure 1.

Table 3 summarizes the results obtained in 1937 with the high-peak type of concentration. The results are very similar to those obtained in 1936 with a low, uniform concentration. The sudden high charge of HCN does not overcome the effects of the stupefying dose of HCN. The red scale remain stupefied for at least 1 hour, as shown by the fact that

fewer scales are killed by the regular fumigation after this interval. Two hours after the scales have been stupefied, they have come out of their stupor and are actually easier to kill; and this is also the case after the 3-hour interval.

TABLE 3

FUMIGATION OF LEMONS INFESTED WITH RESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN SUBLETHAL AND LETHAL CHARGES IN 1937

(Sublethal charge, 0.3 cc of HCN; lethal charge, 12 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of experiments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Probability, P
None	Fumigated	12	9,371	8,050	85.90	− 7.30	0.01
	Stupefied and fumigated	12	9,267	7,284	78.60		
1 hour	Fumigated	12	11,318	10,119	89.41	−11.44	<0.01
	Stupefied and fumigated	12	11,462	8,936	77.96		
2 hours	Fumigated	12	11,419	10,303	90.23	+ 5.85	<0.01
	Stupefied and fumigated	12	12,585	12,091	96.07		
3 hours	Fumigated	11	9,616	8,500	88.39	+ 9.56	<0.01
	Stupefied and fumigated	11	10,387	10,174	97.95		

* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

RESULTS OBTAINED WITH LABORATORY-REARED RESISTANT AND NONRESISTANT STRAINS OF RED SCALE

In August, 1936, the rearing of resistant and nonresistant strains of red scale was begun in insect-proof rooms of the insectary of the Citrus Experiment Station. The original stocks of red scale were collected on August 24 and 25, 1936, from widely separated lemon groves: the resistant strain from Corona, and the nonresistant strain from an isolated grove in the foothills east of Glendora. A series of tests (Quayle, 1938) had definitely shown a wide difference in the susceptibility to HCN of red scale collected from these two groves. In the insectary the red scales were transferred to squash, and the two strains reared in separate rooms under identical conditions.

During the summer of 1937, a method was devised by which crawlers in large numbers were transferred from the stock culture on squash to freshly picked grapefruits. As many as 16 grapefruits were infested daily, 8 with crawlers from the nonresistant stock, and 8 with crawlers from the resistant stock. The grapefruits were held in the rooms in which the stock cultures were kept for several days or until all the crawlers had

settled, after which they were transferred to separate cages in a room in which the temperature was held at 80° F and the relative humidity above 85 per cent. At such high humidity, the grapefruits remained in excellent condition. Approximately 40 days elapsed from the time the grapefruits were infested until the females were mature and started to produce young. In this way a daily supply of red scales of definite age

TABLE 4

FUMIGATION OF LABORATORY-REARED RESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES

(Stupefying charge 0.5 cc of HCN; fumigating charge 4.0 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of experiments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Probability, P
None	Fumigated	7	12,683	10,604	83.61	−13.47	<0.01
	Stupefied and fumigated	7	12,673	8,889	70.14		
1 hour	Fumigated	6	9,413	7,849	83.38	− 8.59	0.01
	Stupefied and fumigated	6	9,894	7,400	74.79		
2 hours	Fumigated	8	13,489	11,122	82.45	+ 4.93	<0.01
	Stupefied and fumigated	8	14,350	12,539	87.38		
3 hours	Fumigated	5	7,631	6,157	80.68	+ 8.37	0.03
	Stupefied and fumigated	5	7,818	6,962	89.05		

* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

could be had for experimental purposes. The date of infesting the grapefruits was marked on each with India ink.

The red scales reared in the laboratory are more homogeneous than those picked at random in the field. Factors which may cause variation such as natural mortality, climatic conditions previous to picking of infested fruit, variation in condition of host plant, age of the scales, and a heterogeneous population of the scales to begin with, are all eliminated when they are reared in the laboratory. Counting of the scales is simplified because all of them on the grapefruit are within a few hours of being the same age; therefore, all the scales on the grapefruit can be counted, whereas fruit picked from the field have scales of all ages. (Scales that are in certain stages only are considered in evaluating fumigation results.)

The experimental procedure with the laboratory-reared insects was the same as that conducted on the red scales obtained from the field. Adult females 40 to 42 days old and just starting to produce young crawlers were used in all the tests. Only the low, uniform type of con-

centration was used, a dosage of 2.0 cc of HCN being used on the nonresistant scales and 4.0 cc on the resistant strain. Since as many as 300 to 450 adults could be reared on a single large grapefruit, only 4 or 5 infested grapefruits were used in each experiment.

The results obtained by the stupefaction and fumigation of the laboratory-reared strain of resistant red scales were similar to those obtained with the same strain from the field. If the resistant red scales reared in

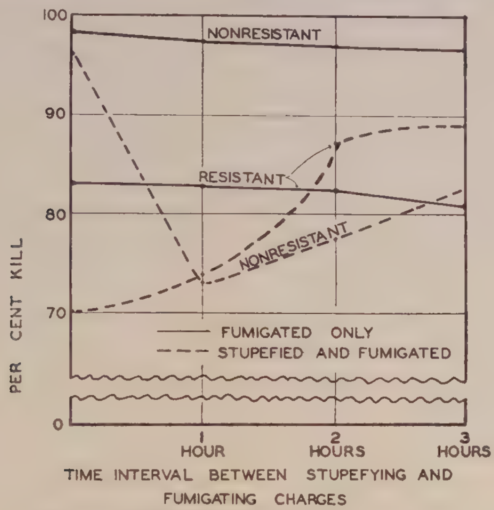

Fig. 2.—Fumigation of laboratory-reared resistant and nonresistant red scale: effects of a stupefying charge. The stupefying charge was 0.5 cc of HCN; the fumigating charge, 2 cc of HCN with the nonresistant strain and 4 cc of HCN with the resistant strain. Data taken from tables 4 and 5.

the laboratory are exposed to a stupefying charge of HCN immediately before, or 1 hour before the regular fumigation, more of them are able to survive the fumigation than those not exposed to the stupefying charge; but if they are exposed to a stupefying charge 2 hours or 3 hours prior to the regular fumigation, fewer are able to survive the fumigation than those not so exposed (table 4 and fig. 2).

It will be noted that 4.0 cc of HCN gave approximately the same kill of the resistant strain of red scale reared in the laboratory as 3.0 cc of HCN gave with the same strain obtained in the field. This may be due to the very favorable conditions under which scales are reared in the laboratory; or continual inbreeding in the laboratory may eliminate nonresistant individuals which are perhaps present in a resistant population in the field.

The nonresistant strain of red scale reacts in an altogether different manner to a stupefying charge of HCN, which seems additional proof of the existence of two strains of the red scale in southern California (table 5 and fig. 2). More of the nonresistant strain are able to survive a normally lethal dose of HCN if they are exposed to a stupefying charge immediately before the regular fumigation; but the difference of the

TABLE 5

FUMIGATION OF LABORATORY-REARED NONRESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES

(Stupefying charge 0.5 cc of HCN; fumigating charge 2.0 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of experiments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Probability, P
None	Fumigated	7	10,478	10,295	98.25	− 2.28	0.01
	Stupefied and fumigated	7	10,498	10,075	95.97		
1 hour	Fumigated	9	17,487	17,083	97.69	−24.08	<0.01
	Stupefied and fumigated	9	17,239	12,690	73.61		
2 hours	Fumigated	9	14,092	13,707	97.27	−19.71	<0.01
	Stupefied and fumigated	9	14,890	11,548	77.56		
3 hours	Fumigated	9	13,475	13,014	96.58	−13.75	<0.01
	Stupefied and fumigated	9	13,362	11,067	82.83		

* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

means is only 2.28 per cent, which indicates that only a few of the nonresistant strain were affected by the stupefying dose. However, if the nonresistant strain of red scale is exposed to a stupefying charge 1, 2, or 3 hours before the regular fumigation, a greater number (24.08 per cent; 19.71 per cent; and 13.75 per cent, respectively), are able to survive the regular fumigation. It appears that the nonresistant strain reacts slower to a stupefying dose of HCN than does the resistant strain. It requires 1 hour before the nonresistant strain becomes fully stupefied, whereas the resistant strain reacts immediately. At the end of 2 hours the effects of stupefaction on the resistant strain are present but operating in another direction, while the nonresistant strain may show the effects of stupefaction in the same direction even at the end of 3 hours.

STUPEFACTION OF OTHER INSECTS

Peters (1936, p. 72) states:

Small traces of hydrocyanic acid cause, in the case of the granary weevil, a shock or stupefying effect that results in cessation of respiration. The hydrocyanic acid can then only enter the body by diffusion, for which high concentrations or long exposures are necessary (therefore the relatively great resistance of granary weevils for hydrocyanic acid) . . . . At lower temperatures, however, the granary weevil, because of a cold phlegma occurring at about 5° C, generally loses the ability to effect this defense reaction, whereby its resistance is lowered. . . .

TABLE 6

COMPARATIVE RESULTS OF FUMIGATION OF GRANARY WEEVIL, CONFUSED FLOUR BEETLE, AND CONVERGENT LADY BEETLE WITH AND WITHOUT STUPEFYING CHARGE

(Stupefying charge, 5 min.; lethal charge, 45 min.)

Insect	Treatment	Stupefying charge, cc	Lethal charge, cc	Number of experiments	Total insects treated	Mean per cent kill	Difference of means,* per cent	Probability, P
Confused flour beetle	Fumigated	....	5.0	6	1,182	84.77	−0.51	0.90
	Stupefied and fumigated	1.0	5.0	6	1,067	84.26		
Lady beetle	Fumigated	....	3.0	7	2,278	82.95	−1.11	0.80
	Stupefied and fumigated	0.5	3.0	7	2,489	81.84		
Granary weevil	Fumigated	....	120.0	6	957	36.47	−19.51	<0.01
	Stupefied and fumigated	10.0	120.0	6	843	16.96		

* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

As a result of their recent experiments, Mackie and Carter (1937) concluded:

One factor not generally considered among those engaged in fumigation of grain is what may be called protective stupefaction, which occurs when a concentration of a gaseous insecticide is not sufficiently strong to kill an insect immediately but knocks it out and causes a suspension of its normal breathing function, thus protecting it against the action of a fumigant.

At the time the experiments were being conducted on red scale, three other insect species, confused flour beetle, *Tribolium confusum* Duval, granary weevil, *Sitophilus granarius* (Linn.), and lady beetle, *Hippodamia convergens* Guérin, were available in numbers large enough for experimental purposes. In all of the experiments with these three species the exposure to the lethal charge of HCN immediately followed the exposure to the stupefying charge.

The data in table 6 indicate that of the three species treated the granary weevil was the only one which definitely showed signs of stupefaction. A reduction in kill of 19.5 per cent was obtained when these insects were first exposed to a sublethal charge of HCN. In the fumigation of stored products "protective stupefaction" may be an important factor, for insects in grain are usually exposed to a low concentration of the gas as it penetrates into the mass of grain.

SUMMARY

Under laboratory conditions with rapid and complete diffusion of hydrocyanic acid gas, a high-peak concentration offers only a slight advantage, if any, over a low, uniform type of concentration on the resistant strain of red scale, *Aonidiella aurantii* (Mask.).

A greater percentage of resistant red scale survive a normally lethal charge of HCN if they have first been exposed to a sublethal dosage of the gas immediately before the regular fumigation.

The effects of the sublethal charge are about the same on the resistant red scale after a 1-hour interval, but after a 2-hour interval the insects have come out of their stupor and are actually easier to kill. They remain easier to kill for 3 hours after they have been subjected to a sublethal charge, but after 4 hours the insects appear to react normally again to a uniform concentration of HCN, that is, as though no stupefaction had occurred.

A sudden high-peak concentration does not overcome the effects of a stupefying charge on the resistant red scale.

Results obtained by the stupefaction and fumigation of laboratory-reared resistant red scale are similar to those obtained with resistant red scale from the field.

Nonresistant red scale require 1 hour before the stupefying charge is effective, as indicated by a large reduction in kill. Nonresistant red scale remain stupefied even after an interval of 3 hours between the sublethal charge and the normally lethal charge of HCN.

The results of this work indicate that there are two definite strains of red scale in southern California.

The granary weevil, *Sitophilus granarius* (Linn.), is readily stupefied by a low concentration of hydrocyanic acid gas, whereas the confused flour beetle, *Tribolium confusum* Duval, and the convergent lady beetle, *Hippodamia convergens* Guérin, are not thus affected.

LITERATURE CITED

Gray, G. P., and A. F. Kirkpatrick.
1929. The protective stupefaction of certain scale insects by hydrocyanic acid vapor. Jour. Econ. Ent. **22**:878–92.

Knight, Hugh.
1925. Factors affecting efficiency in fumigation with hydrocyanic acid. Hilgardia **1**(3):35–53.

Mackie, D. B., and W. B. Carter.
1937. Pest control in rural warehouses and suggested improvements. California State Dept. Agr. Mo. Bul. **26**:275–93.

Moore, William.
1933. Studies of the "resistant" California red scale *Aonidiella aurantii* Mask. in California. Jour. Econ. Ent. **26**:1140–61.

Peters, Gerhard.
1936. Chemie und Toxikologie der Schädlingsbekampfung. 120 p. Ferdinand Enke, Stuttgart.

Pratt, F. S., A. F. Swain, and D. N. Eldred.
1931. A study of fumigation problems: "Protective stupefaction," its application and limitations. Jour. Econ. Ent. **24**:1041–63.

Quayle, H. J.
1938. The development of resistance to hydrocyanic acid in certain scale insects. Hilgardia **11**(5):183–210.

Quayle, H. J., and P. W. Rohrbaugh.
1934. Temperature and humidity in relation to HCN fumigation for the red scale. Jour. Econ. Ent. **27**:1083–95.

Snedecor, George W., and M. R. Irwin.
1933. On the chi-square test for homogeneity. Iowa State College Jour. Sci. **8**:75–81.

4500-6,'38(2219)